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Evaluation of Phenotypic Versus Genotypic Methods Commonly used in the Detection of Oxacillin Resistance in *Staphylococcus aureus*

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Accurate and early detection of methicillin-resistant *S.aureus* (MRSA) is great important for the management of infected patients and select the appropriate infection control measures. Accordingly, evaluation of the accuracy of the phenotypic and genotypic methods commonly used to determine the profile of antimicrobial resistance is essential to ensure that the most appropriate therapy is chosen. Two hundred and twenty eight strains of *Staphylococcus* sp. (106 *S. aureus* and 122 coagulase negative *Staphylococcus* sp.) were used to assess the accuracy of the methods of disk diffusion, oxacillin screening agar and agar microdilution, in comparison with polymerase chain reaction (PCR) for exploring resistance to oxacillin. The *mecA* gene was detected in 31 strains (20.7%), and 29 strains (19.3%) showed discrepant results in at least one of the methods. For *S. aureus*, all the methods showed 100% specificity and sensitivity except for the automated Microscan WalkAway, which showed 92.9% sensitivity and 85%. In relation to coagulase negative *Staphylococcus* sp, the cefoxitin disk had lower accuracy (85% sensitivity). Use of two methods should be the best option for improved sensitivity, offer a time-saving and accurate method of detection of oxacillin resistance in *S. aureus*.

Key words: *Staphylococcus aureus, mecA,* MRSA, Oxacillin, Cefoxitin.

S. aureus is one of the most prevalent pathogens responsible for a wide range of hospital-acquired infections worldwide, particularly in the developing countries[1]. However, coagulase-negative staphylococci (CoNS) have also been common cause of infections, especially in bacteraemia related catheter. Moreover, most of these infections are caused by strains resistant to oxacillin[2]. The World Health Organization (WHO) recognized that antibiotic resistance is one of the major threats facing the world in the future[3]. MRSA is one of the major causes of nosocomial bloodstream infection associated with increased prolonged hospitalization and common cause of morbidity and mortality[2,4]. High rates of isolation of oxacillin-resistant staphylococci cause large-scale use of toxic or expensive antibiotics such as vancomycin. However, in 1996 Japan was the first reported case of vancomycin-intermediate resistance in hospital samples of MRSA and United States in 2002 was the first reported case of full

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resistance to vancomycin, making more evident the hypothesis that these phenomena are associated with the frequent use of this drug[5,6].

As *mecA* gene is not found in staphylococci susceptible to oxacillin, the polymerase chain reaction (PCR) and hybridization-based molecular techniques which determine *mecA* gene are considered to be gold standard methods[7]. The oxacillin resistance is encoded by the *mecA* gene, responsible for the production of a penicillin binding protein (PBP 2a), which has a low affinity for β-lactam agents. The PBP2a acts as a transpeptidase resuming the functions of the cell wall synthesis when other PBPs are inhibited, ensuring the integrity of the bacterial cell in the presence of β-lactam agents[5,8,9].

Several rapid detection methods have been developed for *S. aureus* screening[10,14]. The Clinical and Laboratory Standards Institute (CLSI) indicates the disk diffusion test (DDT), among other methods for determining susceptibility to oxacillin. It also recommends the agar dilution method to quantitatively measure the in vitro activity of antimicrobial agent, with results expressed as minimum inhibitory concentration (MIC). Furthermore, the CLSI indicates the additional confirmatory testing as agar screening, containing 6mg/mL of oxacillin for *S. aureus*, which has shown greater concordance with the direct detection of the *mecA* gene by the PCR method[9, 11,12,13]. Although this method is not suitable for CoNS, some studies have shown that the use of agar screening with 4mg/mL oxacillin showed high sensitivity and specificity in the detection of resistance to oxacillin in CoNS samples, when the method was compared to the PCR method[14,15]. Since 2004, CLSI indicates the use of cefoxitin disk (30 mg) for determining the oxacillin susceptibility in Staphylococcus, because this antibiotic has presented a capacity to induce the expression of PBP2a greater than oxacilina[16]. Several studies have shown excellent sensitivity and specificity of this method for *S. aureus*[16,17,18]. However, for CoNS has been reported a significant reduction in accuracy compared with the method for detection of *mecA* gene by the PCR technique[17,19].

The correct determination of susceptibility to oxacillin in staphylococci samples is crucial because failure to detect this resistance can result in ineffective therapy, leading to unnecessary and indiscriminate use of vancomycin in hospitals. Therefore, the objective of this study was to evaluate the susceptibility of staphylococci to oxacillin samples, comparing the results of oxacillin agar screening methods, DDT with oxacillin and cefoxitin, agar dilution and automated system, with the *mecA* through PCR technique. Most clinical laboratories all over the world depend on disk diffusion testing for the detection of oxacillin resistance *S. aureus* ORSA. Although different methods are recommended by guidelines and validated in multiple studies[20,21], there is a lack of data about the accuracy of these methods. Accurate and rapid identification of ORSA in a clinical specimen is necessary for timely decisions on isolation procedures and effective antimicrobial therapy[4]. The aim of the present study was to determine the accuracy of *mecA* PCR assay to detect methicillin resistance, compared to standard phenotypic susceptibility testing performed using different commonly used methods on the staphylococcal isolates.

MATERIALS AND METHODS

Bacterial strains

In this study, 114 strains (53 *S. aureus* and 61 CoNS) isolated from inpatients at Tanta University Hospital (TUH), Delta, Egypt. *S. aureus* isolates from routine clinical specimens submitted at the microbiology laboratory of the main hospital lab from December 2010 to January 2012 were included in this study. The specimens were obtained from different sites of infection or colonization, the main blood and wound discharge (Table 1). No duplicate isolates from a single patient were included. The identification of *S. aureus* was depended on morphology and biochemical reactions according to standard laboratory criteria (Kloos and Lambe, 1991). Phenotypic characterization to the species level was performed using the API system for the identification of *S. aureus* (Biomerieux, France). To control the investigations, the following standard samples were used: *S. aureus* ATCC 25923, susceptible to oxacillin, and ATCC 33591, resistant to oxacillin. Samples of staphylococci were plated on nutrient agar and incubated for 48 h at 35±2 °C to verify the purity and stored at 20 °C in Tryptic Soy Broth

(TSB) with 20% glycerol.

Genus and species determination of CoNS

Strains were designated as CoNS if all of the following criteria were fulfilled: (i) typical colonies morphology, (ii) Gram positive reaction, (iii) negative tube coagulase test, and (iv) confirmation of the absence of the coagulase gene (*coa*) by PCR. Further species determination was performed using API system. If no exact species could be specified by this examination test system, catalase-positive strains were included as 'non-*S. aureus* strains' in the CoNS group.

Oxacillin susceptibility

Disk diffusion test

The disk diffusion test (DDT) was performed according to the CLSI guidelines[12] with disks of oxacillin (1mg) and cefoxitin (30mg). The diameter of the halo of growth inhibition was performed 24 h incubation at 35±2°C. For the samples of *S. aureus* and CoNS showed that halos sensitivity ≥ 13 mm and ≥ 18 mm for oxacillin disk and ≥ 20mm and ≥ 25mm for the cefoxitin disk were classified as sensitive, respectively. The samples were classified as resistant when presented halos sensitivity is smaller than mentioned[12,13].

Oxacillin agar screening

Samples were subjected to subculture using Mueller-Hinton agar MHA (Difco Laboratories, Detroit, Mich.) containing 4% NaCl and 6µg/ml oxacillin. Standard inocula were prepared from overnight culture in Mueller Hinton Broth MHB (Difco Laboratories, Detroit, Mich.) after matching the 0.5 McFarland (10^5 CFU/ml) and further dilution so as final inoculum is approximately 10^4 CFU/ml. The oxacillin agar plates were inoculated 10 µL of the broth. The plates were incubated at 35±2 ^{0}C for 48 h. The MRSA positive and negative standard reference controls were concurrently used. The growth of one or more colonies per spot indicates positive test.

Latex agglutination test

Latex agglutination test (Denka Seiken Co. Ltd, Japan) was performed according to the manufacturer instructions. For each isolated strain, a 5-µl of *S. aureus* colonies was obtained from fresh subculture and was suspended in micro centrifuge tube containing 200 µl of extraction reagent no. I (0.1 M NaOH). The suspension was boiled for 3-4 minutes and then one drop (50 µl) of extraction reagent no. II (0.5 N KH2PO4) was added and mixed well. The mixture was centrifuged at 2000 × g for 5 minutes at room temperature. Aliquot of 50 µl of the supernatant was added to each of the two circles on a disposable test card and mixed with one drop (25 µl) of the anti-PBP 2a monoclonal antibody sensitized latex and one drop (25 µl) of the negative control latex, respectively. The all contents on the slide were then mixed for 3minutes on a shaker and agglutination was observed visually and was recorded as positive, negative or weakly positive.

Determination of MIC for oxacillin by agar dilution test

MIC was determined for oxacillin by agar dilution method according to the CLSI (2007). Two-fold serial dilutions of antimicrobial agent were prepared in MHA medium. Standardized suspensions of the test organisms (equivalent to the 0.5 McFarland) were prepared from overnight cultures in MHB. The test organisms were inoculated in approximately 10^4 CFU/ml. The plates were incubated at 35±2°C for 24 h. Reference quality control organisms were used. Samples were inoculated on Mueller-Hinton plates supplemented with 2% NaCl containing oxacillin in concentrations ranging from 0.0625 to 1.024µg/mL. Were considered oxacillin resistant samples *S. aureus* and CoNS showed that CMI ≥ 4mg/mL and ≥ 0.5mg/mL[13] respectively.

Detection of the *mec* gene by PCR

DNA extraction

Bacterial DNA was extracted according to the method previously described by Ida *et al*. 2001. Briefly, colonies obtained from overnight *S. aureus* cultures from sheep blood agar were harvested and suspended in 100 ml of lysis solution (20 mM Tris HCl, 140 mM NaCl, 5 mM EDTA [pH 8.0]). Three units of lysostaphine were added and the suspension was incubated at 37ºC for 3 hours. 200 ml of distilled water was added and incubated at 95ºC for five minutes. Phenol-chloroform extraction and ethanol precipitation steps were then performed for DNA extraction.

PCR amplification

PCR amplification was carried out on purified genomic DNA extracted from clinical isolates. Each DNA sample (10 µl) was added to 90 ml of the PCR mixture consisting of 10x Buffer, 25mM of $MgCl_2$, 2.5U of Taq polymerase, 0.25mM of dNTP and 0.5µM of each primer; (5 '- TAG TGA

AAA CTG AAC GTC GC - 3') and MRS2 (5 '- CGA TTG TCA ATG TTA TAG CCG - 3') used to detect a segment of 154pb *mecA* gene (encoding resistance to oxacillin) (21). The amplification conditions were: Initial denaturation was carried out for 3 mins at 92°C followed by 30 cycles of amplification (denaturation at 92°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min with an increment of 2 secs per cycle). The final extension was performed at 72°C for 3 mins. Amplification was carried out in a Bio-Rad thermal cycler. After amplification, 17 µl of PCR sample was loaded on a 1 % (w/v) agarose gel (Bioline, London, UK) containing 0.5 gr/ml ethidium bromide and run in a horizontal gel electrophoresis unit (Mini-Sub DNA cell, BioRad). The running buffer was TAE [40 mM Tris, 20 mM acetic acid, 1 mM ethylene diamine tetra-acetic acid (EDTA), pH 8.0]. Electrophoresis was carried out at 100 V for 2 h on an Amersham- Pharmacia Biotech (Uppsala, Sweden) power supplier unit ECPS3000/150. The stained bands were visualized with UV light (309 nm) using a trans-illuminator and gels were recorded as digital TIFF images using a gel documentation system (UVI-Tech).

Statistical analysis

Results were statistically analyzed using SPSS (version 13, SPSS, Chicago). The analysis was depending on the differences in susceptibility of the used methods and the significance of the results were calculated by the Chi-square test. The *P* value of < 0.05 was considered to be statistically significant. Validity tests including susceptibility, specificity, positive predictive value, and negative predictive value were calculated. Susceptibility was defined as the percentage of *mecA*-positive isolates determined to be susceptible by phenotypic testing and specificity was defined as the percentage of *mecA*-negative isolates determined to be susceptible by phenotypic testing. The 2007 CLSI guidelines were used to determine susceptibilities.

RESULTS

The *mecA* gene was detected in 48 (42.1%) samples (13 *S. aureus* and 35 CoNS). Eighty-seven (76.3%) samples showed agreement in the results of the methods evaluated in relation to the gold standard, while the remaining 27 samples showed discrepant results in at least one of the methods, 20 (74.1%) CoNS and 7 (25.9%) *S. aureus* (Table 2).

Whereas, the sensitivity and specificity of a method results are truly related to positive and negative, respectively, when compared to a standard method, our results showed 100% sensitivity and specificity in all methods for samples of *S. aureus* evaluated, except for the automated Microscan WalkAway, which showed 92.9% sensitivity and 85% specificity (Table 3). Values lower sensitivity and specificity (77.1% and 84.6%, respectively) were also obtained using the automated system for CoNS. The other methods had greater variation in sensitivity and specificity for the CoNS than observed with samples of *S.*

Table 1. Distribution of species and sites of isolation of 114 strains of staphylococci.

Species	Samples No (%)	Sites of Isolation No of samples
CoNS	61 (53,5 %)	
Staphylococcus epidermidis	37	Blood (18) Catheter tip (6) secretion (11) Urine (2)
S. hominis	7	Blood (6) Catheter tip (1)
S. haemolyticus	6	Blood (3) Catheter tip (1) secretion (2)
Staphylococcus spp	3	Blood (2) Secretion (1)
S. xylosus	2	Blood (2)
S. auricularis	1	Secretion (1)
S. sciuri	1	Secretion (1)
S. saprophyticus	1	Urine (1)
S. cohnii	1	Blood (1)
S. warneri	1	Blood (1)
S. capitis	1	Blood (1)
Staphylococcus aureus	53 (46,5 %)	Blood (17) Catheter tip (4) secretion (29) Urine (3)
Total	114	Blood (51) Catheter tip (12) secretion (45) Urine (6)

Table 2. Results of 27 samples of staphylococci that had discrepancies in one or more phenotypic methods in relation to the PCR method (gold standard)

Species	PCR*mecA*	DDT		Agar dilution		Oxacillin agar screening 4µg		Oxacillin agar screening 6µg		Microscan WalkAway	Latex agglu tinationtest
		CFO	OXA	24h	48h	24h	48h	24h	48h		
Staphylococcus aureus	N	S	S	0,5	1	-	-	-	-	R (> 2)	-
S. aureus	N	S	S	0,25	0,5	-	-	-	-	R (> 2)	-
S. aureus	N	S	S	0,5	0,5	-	-	-	-	R (> 2)	-
S. aureus	N	S	S	0,5	0,5	-	-	-	-	R (> 2)	-
S. aureus	N	S	S	0,5	1	-	-	-	-	R	-
S. aureus	P	R	R	128	128	+	+	+	+	S (0,5)	+
S. aureus	N	S	S	0,5	0,5	-	-	-	-	R (> 2)	-
S. epidermidis	N	S	S	<0,0625	0,125	-	-	-	-	R (> 2)	-
S. epidermidis	N	S	S	0,25	0,25	-	-	-	-	R (> 2)	-
S. epidermidis	P	S	R	8	32	+	+	+	+	R (> 2)	+
S. epidermidis	P	S	R	1	2	+	+	+	+	S	+
S. epidermidis	P	S	R	1	2	+	+	+	+	R (> 2)	+
S. epidermidis	P	S	R	8	8	+	+	+	+	R (> 2)	+
S. epidermidis	P	S	R	32	32	+	+	+	+	R (> 2)	+
S. epidermidis	P	S	S	0,125	0,5	+	+	+	+	R (> 2)	+
S. hominis	P	R	R	32	64	+	+	+	+	S (d"0,25)	+
S. hominis sub hominis	N	S	S	1	1	-	-	-	-	R	-
S. hominis sub hominis	N	S	S	<0,0625	0,125	-	-	-	-	R (> 2)	-
S. hominis sub hominis	P	R	R	8	8	+	+	+	+	S (< 1)	+
S. xylosus	N	S	S	0,25	0,25	-	-	-	-	R (> 2)	-
S. xylosus	N	S	S	<0,0625	<0,0625	-	-	-	-	R (> 2)	-
S. sciuri	N	S	S	1	2	-	-	-	-	S (d" 0,25)	-
S. haemolyticus	P	R	R	1.024	1.024	+	+	+	+	S (d" 0,25)	+
S. haemolyticus	P	R	R	512	1.024	+	+	+	+	S (d" 0,25)	+
S. auricularis	P	R	R	64	128	+	+	+	+	S (d" 0,25)	+
CoNS	P	S	R	1	2	+	+	+	+	R (> 2)	+
CoNS	P	S	R	2	2	+	+	+	+	R (> 2)	+

CFO: cefoxitin, OXA: oxacillin, DDT: disk diffusion Test, R: resistant S: susceptible, +: growth of one or more colonies, -: no growth, P: mecA positive, N: negative mecA, CoNS: coagulase negative *Staphylococcus*. Limits of resistance to oxacillin: *S. aureus* CMI CMI ≥ 4 µg/mL CoNS and ≥ 0.5 ug / mL (CLSI, 2007).

aureus, due to the presence of one to eight samples with discrepant results (Table 4).

DISCUSSION

The genus Staphylococcus is considered of great importance due to its high prevalence in nosocomial infections, in addition to presenting high rates of resistance to oxacillin and other antimicrobials complicating the treatment of patients. It is a major cause of serious hospital and community-acquired infections associated with morbidity and mortality rates with rapid development of resistance[22]. Currently, one of the main objectives for the control of hospital infections is the rational use of antimicrobials, which makes the evaluation of the accuracy of the phenotypic methods used for determining the susceptibility profile essential to ensure the most appropriate choice of antimicrobial treatment[23,24].

Table 3. Results of susceptibility to oxacillin among the 53 samples of *S. aureus* and calculation of sensitivity and specificity of phenotypic methods in comparison with the detection of the *mecA* gene by PCR method

Method	No. of Samples				Sensitivity (%)	Specificity (%)
	mecA positive*mec* (No= 13)		*A* negativo (No= 40)			
	resistant	false-resistant	resistant	false-resistant		
DDT oxacilin	13	-	40	-	100,0	100,0
DDT cefoxitin	13	-	40	-	100,0	100,0
Agar dilution	13	-	40	-	100,0	100,0
Microscan WalkAway	12	6	30	1	92,9	85,0
ASOx 4µg/mL (24h)	13	-	40	-	100,0	100,0
ASOx 4µg/mL (48h)	13	-	40	-	100,0	100,0
ASOx 6µg/mL (24h)	13	-	40	-	100,0	100,0
ASOx 6µg/mL (48h)	13	-	40	-	100,0	100,0
Latex agglutination	13	-	40	-	100,0	100,0

DDT: disk diffusion test, ASox: oxacillin agar screening, sensitivity: percentage of *mecA*-positive samples classified correctly, specificity: percentage of *mecA*-negative samples correctly classified.

Table 4. Results oxacillin susceptibility between CoNS 61 samples and calculation of sensitivity and specificity of phenotypic methods compared to the detection of the *mecA* gene by PCR method

Method	No. of Samples				Sensitivity (%)	Specificity (%)
	mecA positive*mec* (No= 13)		*A* negativo (No= 40)			
	resistant	false-resistant	resistant	false-resistant		
DDT oxacilin	34	0	26	1	97,1	100,0
DDT cefoxitin	27	0	26	8	77,1	100,0
Agar dilution	34	2	24	1	97,1	92,3
Microscan WalkAway	27	4	22	8	77,1	84,6
ASOx 4µg/mL (24h)	34	1	25	1	97,1	96,1
ASOx 4µg/mL (48h)	25	1	25	-	100,0	96,1
ASOx 6µg/mL (24h)	34	-	26	1	97,1	100,0
ASOx 6µg/mL (48h)	25	1	25	-	100,0	96,1
Latex agglutination	34	0	26	1	97,1	100,0

DDT: disk diffusion test, ASox: oxacillin agar screening, sensitivity: percentage of *mecA*-positive samples classified correctly, specificity: percentage of *mecA*-negative samples correctly classified.

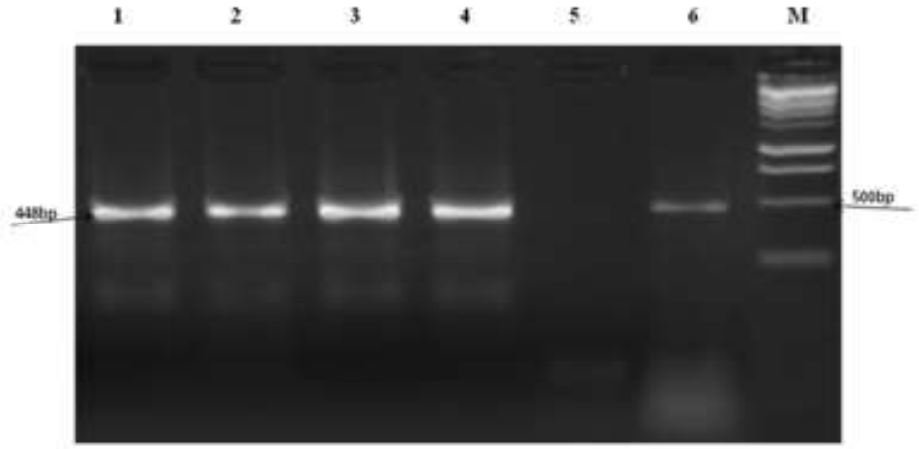

Fig. 1. Gel image of representative PCR *mec* gene products, *mecA* (448 bp), Lanes 1- 4; *mecA* positive (strain numbers 15, 57, 130 and 184), Lane 5; *mecA* negative control, Lane 6; *mecA* positive control (448 bp) and Lane M; 1 kb DNA ladder

However, some samples of Staphylococcus (especially CoNS) oxacillin resistant feature heterogeneous expression of resistance, i.e., a colony forming unit (CFU) between 10^4-10^8 CFUs expressed phenotypic resistance[25]. These few resistant cells may lead to false-negative results on conventional methods for determining susceptibility and can be selected in patients receiving treatment with ²-lactams, thus leading to failure treatment[13]. In this study, six different phenotypic methods were compared with *mecA* PCR, especially the automated system and DDT are the methods most commonly used by microbiological diagnostic laboratories in Egypt.

To *S. aureus*, almost all methods showed good correlation with the PCR method, showing 100% sensitivity and specificity after 24 hours of incubation, except for the automated Microscan WalkAway. Some researchers showed high level of accuracy of these methods for this species[11,15] where the heterogeneous expression of resistance did not significantly influence the experimental conditions as 24 h incubation at 35±2°C, in the absence of NaCl DDT and low inoculum used in the method of agar dilution (~10 CFU/mL)[5]. However, the results of this study also showed that six samples of *S. aureus mecA*-negative results showed false resistance and *mecA*-positive sample was considered susceptible to oxacillin by WalkAway Microscan automated method (92.9% sensitivity and 85% specificity). These results may be related to the high expression of ²-lactamases in *mecA*-negative or, in the case of *mecA*-positive sample, a heterogeneous expression of oxacillin resistance.

Regarding CoNS isolates, a greater number of discrepant results has observed compared to *S. aureus* samples. Latex agglutination, DDT and ASox as oxacillin yielded better sensitivity and specificity compared to PCR (Table 2). Although a sample of *S. sciuri mecA*-negative have been able to grow in ASox at 48h in both concentrations (false-positive result for resistance), incubated for another 24 h reversed the false-negative results observed for a sample of *S. hominis mecA*-positive. Some studies have shown the method ASox 4mg/mL with oxacillin as the best choice for confirmation of oxacillin resistance in the CoNS[14,13]. The lowest concentration and extended incubation period (48 h) avoid false-negative results due to frequent feature hetero-resistant CoNS mainly in the species *S. epidermidis*[14,26,15]. A study achieved by Baddour *et al*.[27] revealed that PBP2a latex agglutination test methods were more sensitive than cefoxitin and oxacillin disk-diffusion methods, but cefoxitin disk diffusion was the most specific. In addition, they found that a combination of oxacillin disk diffusion with latex agglutination test improved sensitivity and specificity and concluded that the use of more than one screening method is important to detect all MRSA isolates in clinical settings.

Regarding DDT oxacillin, only a sample of *S. epidermidis mecA*-positive were sensitive resulting in a value of 97.1% sensitivity and 100% specificity. In spite of having 100% specificity, the sensitivity of cefoxitin disk was lower than for oxacillin (77.1%). This result may be an indication that the cefoxitin disk should not be used alone to predict resistance to oxacillin mediated by PBP2a in samples of CoNS. These results corroborate other studies that showed inferiority or equivalence in sensitivity or specificity of oxacillin compared with cefoxitin, indicating that the performance of DDT cefoxitin is not as good as for *S.aureus*[25, 28,29]. However, in this study the combined results of the two albums generated a value equal to the sensitivity of the oxacillin disk alone (97%) due to a sample of *S. epidermidis mecA*-positive have been sensitive to both antibiotics. The same was observed by Perazzi and cols[29] who found a significant increase in sensitivity (90%) and specificity (100%) in the combination of the two disks.

Discrepant results occurred in 23.7% of samples, and CoNS most (77.8%) of these. This reflects the difficulty in determining susceptibility to oxacillin some samples of CoNS, mainly due to a heterogeneous expression of resistance which contributes to the false-susceptibility results. This feature should be considered in the choice of methods to be used for routine monitoring. For example, the inoculum agar dilution method (10^4 CFU / ml) is significantly smaller than the ASox (10^7 CFU / ml). Moreover, the incubation time of 24h for both DDT and determining MIC can be insufficient for growth of the strains hetero-resistant that are fewer in number (one for each 10^6 CFU / mL).

The Microscan WalkAway automated method is often used in routine diagnostic laboratories microbiological large and showed lower accuracy compared to other methods, with the largest number of discrepant samples (18 of 27 samples). This method proved false resistance results for 12 (44.4%) samples and sensitivity to false 6 (22.2%) samples. A disadvantage of this system is the use of a low number of concentrations of oxacillin generally limited to concentrations as defined breakpoints set in CLSI (concentrations ranging from 0.25 to 2 mg/mL). The association of the automated system, another method should be adopted by microbiological diagnostic laboratories to ensure increased accuracy in the determination of resistance to oxacillin.

Despite the routine diagnostic microbiological laboratories is a race against time, it is important to conduct the evaluation of the accuracy of methods for detection of oxacillin resistance in staphylococci. The use of two methods should be the best option for improving the accuracy. Ex: a combination of automated methods with DDT, DDT with cefoxitin and oxacillin or DDT with ASox. These associations had virtually 100% sensitivity and specificity in our study and are low cost. Thus, the safety and quality of results are guaranteed and therefore avoid unnecessary use of vancomycin or treatment failure. Use of two phenotypic methods is also recommended by Kaiser *et al*.[30] in order to improve accuracy, especially when a diagnostic laboratory only uses an automated system or oxacillin disk diffusion test.

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